CHROMOSOME MOVEMENT IN A HAPLOID OF *TRITICUM AESTIVUM* L.

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CHROMOSOME MOVEMENT
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TABLE OF CONTENTS

ABSTRACT

Bivalents, univalents, end-to-end and side-to-side associations, and also chromosome distribution to the plate and poles were recorded for each of approximately 2000 meta-anaphase cells of a haploid of *Triticum aestivum* L. The determination of non-randomness in univalent distribution and of an inverse relation between side-by-side association and bivalent frequencies formed the first in a series of analytical steps leading to a hypothetical description of chromosome movement in the haploid. Using statistical methods, the hypothesis was tested and found to agree with the observations. Further cytological evidence was advanced in favor of the hypothesis. The role of the spindle was considered and the operation of polar forces during late prophase was suggested. The possible action of these forces was described. Conclusions relating to the general problem of haploidy were outlined.

INTRODUCTION

> " ..... there is every likelihood that the behavior of several elements of the cell may be analyzed separately, and indeed it is because of this possibility that we may have hope of a final solution to the problem of mitosis." Franz Schrader, "Mitosis", 1944.

The movements of the chromosomes during cell division have been the object of many studies in the past, and as a consequence, many hypotheses designed to explain chromosome movement have been proposed. These have been recently reviewed by Schrader (46). He concludes that none of the many hypotheses has in it definite promise of a final solution, and that the solution, when available, will have important applications in all fields of biological research.

Factors important to the normal and harmonious functioning of biological systems and processes have often been revealed by investigating the system or process in an abnormal, rather than a normal condition. To apply the same approach to the movements of chromosomes in the mitotic process would require organisms able to maintain mitotic activity

under abnormal conditions, or with an abnormal mitotic apparatus. Cytologists have preferred to study organisms with few chromosomes, and investigations have been, therefore, largely confined to diploids whose tolerance to abnormality is low. Of the organisms with a high tolerance to abnormality in chromosome behavior, common wheat (Triticum aestivum L.) is perhaps best known. It maintains meiotic and mitotic functioning when up to half its chromosomes are missing, as in haploids, and also when foreign chromosomes are introduced, as in interspecific and intergeneric hybrids, and thus presents a wide range of abnormal chromosomal activity for study by the analytical cytologist.

REVIEW OF LITERATURE

Although haploidy was first demonstrated in Datura in 1922 (4) plants had been described earlier that were undoubtedly haploid (15). In 1924 a haploid was discovered in Nicotiana (8) and in 1926 another in Triticum (11). The three genera in which these haploids were first found have continued to

provide the majority of haploids (25); by 1937 over 200 had been recorded in Datura alone (44). Others have been found in more than twenty genera (6, 9, 14, 15, 17, 18, 21, 22, 23, 25, 26, 29, 30, 32, 35, 37, 38, 40, 42, 49, 50, 54, 58). Reviews of the literature on haploidy have been written by Bleier (6), Ivanov (21), Kostoff (25) and others.

Haploid mitosis has been described by several authors (6, 17, 18, 21, 25, 44, 54). Cell division is usually regular although chromosome doubling frequently occurs (11, 17, 18, 44, 54). There have been many more descriptions of haploid meiosis (5, 6, 11, 14, 18, 23, 25, 26, 28, 29, 30, 32, 35, 37, 38, 42, 49, 50, 54, 58). Chromosome behavior in each haploid is unique. There is variability from haploid to haploid within a species (25, 27) and from cell to cell within any haploid (5, 6, 11, 14, 18, 23, 25, 26, 28, 29, 30, 32, 35, 37, 38, 42, 49, 50, 54, 58). In this review it was considered more convenient and more useful to describe events common to all or most haploids than to attempt to catalogue all events in every haploid.

Prophase is difficult to study in haploid meiosis (6, 9, 21, 25, 26, 38). This may be because of poor fixation associated with the haploid condition (38) but usually it is because the bivalents, which normally provide the morphological indices for recognizing the prophase stages, are absent (5, 23, 25). Chromosome pairing at prophase does not occur in some

haploids (5, 6, 23, 25), but usually one or more paired segments can be seen (6, 9, 21, 25, 58); in haploid Secale all chromatids are paired at prophase (30). Pairing may occur between homologous chromosome parts - as determined by minutely examining the paired chromomeres (25) - or between non-homologous chromosomes (30, 31). At a stage in early prophase comparable to diplotene in normal meiosis the chromosomes appear double (23, 25, 42). By late prophase the chromosomes are contracted and they appear single (6, 9, 21, 25, 58).

At late prophase three kinds of chromosome associations are observable in haploids:

(i) Bivalent and multivalent associations with chiasmata. The bivalents are characteristically open ("rod-bivalents"), often heteromorphic, with the single chiasma usually terminalized (6, 9, 11, 14, 21, 23, 25, 26, 32, 38, 40, 49, 50, 58). Closed bivalents, trivalents and quadrivalents are only occasionally seen (6, 9, 21, 25, 26, 30, 32, 58).

(ii) Chromosomes aligned side-by-side in twos. The distance between them may be as much as half the diameter of a chromosome (6, 9, 13, 16, 21, 25,

28, 32, 33, 49). The members of the association are usually, though not always, equal in length (9, 13, 16, 25, 28). This kind of association has been variously described as "secondary pairing" (9, 25, 33, 49), "secondary association" (13, 16, 28), "pseudo-gemini" (13) and "residue pairing" (30). In the present study they are referred to as "s-s associations".

(iii) End-to-end association of two or more chromosomes. The members of the association appear to be in physical contact at each junction. An association may include the entire chromosome complement (23) but most commonly it is formed of two chromosomes (13, 21, 23, 25, 58). They have been described as "tandem associations" (25) and "end-to-end associations" (23). The latter is abbreviated to "e-e associations" in this study.

In some haploids none of these associations occurs (5). In some of the papers reviewed they can be seen in the illustrations though they are not mentioned in the text (11, 25, 54). In other haploids one or all of the associations may be found, sometimes all within a single cell (6, 9, 13, 16, 21, 25, 28, 32, 33, 49). E-e associations occur frequently

in cells of haploid T. monococcum (23, 24, 28); bivalents and s-s associations are more numerous in haploid T. aestivum (T. vulgare) (6, 11, 21, 25, 26, 32, 40, 58). In general there are more bivalents formed in haploids of suspected polyploids than in haploids of "good" diploids (6, 9, 21, 25, 40). With respect to e-e and s-s associations no generalizations have been made.

It is generally agreed that bivalents are produced during early prophase through crossing-over and chiasma-formation within homologously paired chromosome segments (3, 6, 7, 9, 21, 25, 31, 34, 45, 46, 49). There is no agreement as to when the other associations appear. Levan (30) and Heilborn (16) believe they are formed during early prophase, others (9, 13, 25, 33) that they first appear at late prophase. By the end of prophase variable numbers of univalents, bivalents, e-e and s-s associations are scattered randomly throughout the cell (5, 6, 11, 14, 18, 21, 23, 25, 26, 30, 35, 38, 46, 50, 58).

Few authors describe the transition from late prophase to metaphase. Krishnaswamy reports that there is "sudden entry" into metaphase (26). Hakansson says that the chromosomes "ordnen sich schnell zu einer Metaphaseplatte" (14). The present author agrees that the transition is rapid.

At metaphase the bivalents, when present, are found at the equatorial region of the cell; the univalents and remaining associations are found usually in the polar regions (6, 9, 11, 14, 21, 25, 32, 40, 54, 58). In haploid cells containing no bivalents it is not possible to define a metaphase stage, and such cells are usually considered to be at anaphase (5, 11, 23, 26, 32, 38, 49, 50). Tometorp states that no boundary can be drawn between metaphase and anaphase and uses the term "meta-anaphase" to describe the stage between late prophase and telophase (50). The term is also used in the present study.

At meta-anaphase the chromosomes are usually hypercontracted (25). This is seen in the illustrations of most authors. In one case they are described as "elongated" (26).

The number of e-c associations is considered by some authors to decrease from prophase to meta-anaphase (23, 30). S-s associations may increase (19, 13, 16, 25) or decrease (30) but in no report is the observation supported by numerical data.

Univalents at the meta-anaphase plate have been observed in several haploids (6, 11, 14, 25, 26, 38, 50). They divide only rarely during meta-anaphase (6, 11, 14, 25, 26, 38, 50).

Spindles at meta-anaphase are only occasionally mentioned. Two authors, Hakansson (14) and Krishnaswamy (26), report that the shape of the spindle is influenced by the position of the chromosomes, becoming "bent" or "half-moon" in shape in certain cells. A crescent-shaped spindle had been reported earlier (11).

Distribution of the chromosomes to the poles is usually random. Observed and expected distribution frequencies were compared by several authors (5, 6, 23, 25, 32, 58) and in only two instances the distribution was not random (23, 32). Univalent movement in haploids has been compared with that in triploids (5, 9, 25, 27, 36). Many authors state that the distribution of chromosomes in haploids is random without presenting numerical data (11, 14, 26, 35, 38, 54). When the haploid cell contains bivalents the separation of the bivalent-halves is usually normal (6, 11, 14, 21, 25, 26, 38, 50, 54, 58) although anaphase bridges are occasionally seen (49, 50).

Throughout most of meta-anphase the chromosomes remain single. When doubling does occur, at late meta-anaphase or telophase, it is shown by all the chromosomes present (6, 11, 14, 18, 21, 25, 26, 38, 50, 54, 58). The following description is taken from Gaines and Aase (11),

"When the lengthwise splitting is initiated it progresses almost simultaneously in all the chromosomes of the cell, and it is probable that, if a dividing chromosome lies near the center of the spindle, its halves are more likely to separate than if it lies nearer to the pole". In only a few cells have univalents been seen dividing at the plate at late meta-anaphase (11, 26, 38, 50). Hakansson states, "only very seldom does a centromere divide before interkinesis" (14, English summary).

There has been no clear definition as to when telophase begins in haploid cells. The observation common to all telophase cells is the appearance of doubleness in the chromosomes (5, 11, 14, 23, 25, 26, 38, 50). This statement includes, of course, the haploid cells in which doubleness was reported at late anaphase (see above). For the purposes of the present study the beginning of telophase is defined as the time at which the chromosomes appear double. For the chromosomes only the changes throughout telophase and interkinesis are comparable to those in normal meiosis. The chromosomes become more tightly grouped, more diffuse, and form a spherical nucleus (6, 11, 14, 26, 38, 50). With few exceptions (50) wall formation occurs (6, 11, 14, 25, 26, 38).

Restitution and "dumbell" nuclei have been observed (11, 14, 25), and also chromosomes "trapped" in the cell wall (26), but most commonly two unequal nuclei, with occasionally micronuclei, are formed (6, 11, 14, 18, 21, 25, 35, 37).

During second meiosis there is division of all chromosomes (5, 14, 23, 25, 26, 38, 50), except in those cells where univalents have divided at first meiosis; in these they may interfere with the normal anaphase movement of the remaining chromosomes (11). The most comprehensive description of the action of the spindle at second anaphase is as follows, "spindles are formed quite irrespective of the number of chromosomes, but the size of the spindle depends on the number. Without exception the spindles formed are bipolar. Sometimes weak attempts at forming spindles about micronuclei, whose chromosomes have not even made an attempt to go into the metaphase condition, are found" (26).

Restitution and formation of micronuclei may again occur (11, 14, 21, 25, 26, 35, 38, 50). Following wall formation one or more nuclei may be contained within a single spore (6, 11, 14, 21, 25, 36, 38, 50, 54). Pollen formation is usually abortive and few grains are functional; degeneration may begin at anaphase II (38) but more usually it begins later (5, 6, 11, 14, 23, 25, 26, 50).

Other interesting observations not of general occurrence in haploid meiosis include fusion of microsporocytes during prophase (29) and syncytial metaphases with up to 150 bivalents and univalents forming a single large plate with one large spindle (11, 29). Also several investigators have reported that the extent of pairing and chiasma formation in haploids is markedly influenced by external conditions (6, 25, 28, 54); the same has been shown for diploids (9, 12, 21, 40, 55).

One of the chief problems of haploid meiosis concerns the possible role of homology in determining the movements of the chromosomes during prophase and meta-anaphase. This has been especially difficult since Levan (30) has so clearly shown that non-homologous pairing occurs in haploid rye. He states, "These observations on the haploid pachytene pairing make it absolutely indisputable that non-homologous pairing plays a dominant role". While this and a previous (31) observation do not negate the importance of homology in chiasma and bivalent formation, they provide, as Levan has shown, mechanisms whereby e-e and s-s associations may be produced through the action of relational coiling. Smith's attempt to determine the importance of homology in secondary pairing was inconclusive (49). He states "those

who believe in secondary pairing will form a different opinion from those who think that secondary pairing is an artifact or that it results from cytoplasmic factors independent of homologies between the chromosomes." Other authors have also studied this important problem (6, 9, 13, 16, 21, 24, 25, 26, 28, 32, 33, 40). Lammerts (28), who first described e-e associations, did not believe they resulted from homologous attraction. Kostoff (24) demonstrated terminal heterochromatin in the chromosomes of T. monococcum and suggested that e-e association results from non-specific attraction. Heilborn (16), who described s-s associations in 1924, considers that they result from the mechanical sorting out of chromosomes according to their size. Kostoff (25) believes that a small number of homologous genes, when concentrated within a small volume through chromosome coiling, may be sufficient to bring about homologous attraction between chromosomes not previously paired. Gustafsson (13) and Matsuura (cited by Kostoff, 25) believe that the association is maintained through fusion of the chromosome "sheaths" or "pellicles". Others consider that the associations are merely the result of abnormal prophase (26).

The formation of e-e and s-s associations and their subsequent behavior are considered in the present study.

MATERIALS AND METHODS

The haploid described in this study was found in a true-breeding mutant line derived from Triticum aestivum L. var. Thatcher. The mutation was induced at the University of Saskatchewan using ionizing radiation (1). Cytological studies showed that one tiller of the plant was haploid and two were diploid. Of four remaining tillers two were sterile and two were fertile. The plant was, therefore, a haplo-diplo chimaera.

The spikes chosen for cytological examination were fixed for two days in Carnoy's solution B. The anthers were then removed and cut transversely on a slide in a drop of aceto-carmine dye, a coverslip was then applied and tapped gently until the anther and its contents were fairly evenly dispersed. This preparation was heated gently over an alcohol flame until small air-pockets began to appear beneath the coverslip and then pressed firmly between the halves of a folded blotter. Following this the coverslip was floated off in Farmer's fluid and remounted using a small drop of Euparol. The slides were stored for a week at room temperature before being examined under oil immersion.

Microscopic observations and photographs were made using a Zeiss Winkel Opton microscope fitted with apochromatic lenses and a photographic attachment.

Since preliminary observations had revealed a variety of chromosome arrangements, with a variable behaviour for each, a full list of all arrangements and associations, together with their range of variability, was compiled. These were recorded, together with the distribution and behavior of univalent chromosomes, for each cell, in such a way that a correlation of the behavior of any type of chromosome or chromosomal arrangement could be correlated with that of any of the others. For doing this a taxonomic system of scoring was devised: The cells were first classified according to the numbers of bivalents which were present; these were grouped depending on the numbers of bivalents with and without chiasmata, and subgrouped according to the distribution of the chromosomes to the poles. The number of chromosomes associated side-by-side or end-to-end (s-s and e-e associations), or occurring as univalents or in complex associations (trivalents, quadrivalents) at the plate was recorded for each individual cell. Detailed descriptions of these phenomena will appear later; they are mentioned here to more conveniently illustrate the methods used in scoring.

OBSERVATIONS

Early prophase is difficult to study even in cells with few chromosomes. In wheat the chromosome number is comparatively large and it is rarely possible to follow the paired chromosomes for more than a small fraction of their total length. It is usually possible, through studying the disposition of the chiasmata, to determine the stage in prophase which the cell has reached. In the haploid wheat cells there were few or no chiasmata and reliable determinations of the division stage could not be made. Detailed studies of the behavior of individual chromosomes could not be undertaken.

At diakinesis only a few cells were available for study. The chromosomes were distributed throughout the nucleus; their edges were not as clearly defined and their morphology was more irregular than at later stages. The cell in figure four shows some of these characteristics. Since this cell has no nucleolus it is probably at a stage slightly later than diakinesis, perhaps at the stage corresponding to prometaphase in normal meiosis (fig. 1).

Following diakinesis there was movement of the chromosomes to the plate and poles. The term "meta-anaphase" is used to describe cells in which these movements have taken place.

In meta-anaphase cells the bivalents, when present, were situated in the equatorial region of the cell with their daughter-halves oriented to the poles (figs. 5-15, 18-21, 23). The position and orientation of the bivalents was therefore not significantly different from that in normal metaphase and early anaphase cells (figs. 2, 3). The spindle-attachment fibres which are usually seen during normal meta- and anaphase (figs. 2, 3) and occasionally at prometaphase (fig. 1) were very seldom distinguishable in bivalents of haploid cells. The "secondary split", observable in chromosomes at early normal anaphase (fig. 3) was not observed in any haploid meta-anaphase cell. Dividing bivalents in the haploid did not show the rather sharp bending at the kinetochore which is characteristic of normal anaphase (fig. 3); this may be a property of open bivalents in both haploid and normal cells. The characteristics described here for bivalents are shown in figures five to fifteen, eighteen to twenty-one and twenty-three.

As bivalent daughter-halves moved to the poles during meta-anaphase the chiasmata became progressively

smaller and disappeared, leaving the chromosomes connected by a slender chromatin thread (fig, 7). With further separation the thread was no longer visible (fig. 13). As in normal anaphase (fig. 3) the separation of bivalent-halves was not always synchronous (figs. 6, 19-21). The number of bivalents with chiasmata and with daughter-halves connected by threads was recorded for each cell. In a later analysis it was considered that cells with bivalent-halves connected by threads are at a later stage than those with all bivalents showing chiasmata.

In meta-anaphase cells the univalents were usually found in the polar regions (figs. 5, 8, 13, 18) although a univalent was occasionally seen near the equator (figs. 6, 7, 9, 15, 18, 21, 23). Polar grouping of univalents was also seen in cells without bivalents (fig. 24). In all cells it could be seen that the movement of univalents in forming the polar groups, had been in a direction perpendicular to the "metaphase" plate. This was probably a manifestation of cell polarity (52). The orientation of univalents within the polar groups was haphazard (figs. 5-9, 12-13, 15, 18-20). Occasionally univalents were found in the equatorial region of the cell. They were usually oriented either in line with the polar axis (figs. 7, 9) or in line with the metaphase plate (figs. 15, 23), but there were cells in which these orientations were not seen (fig. 18).

Closed bivalents, and also trivalents and quadrivalents were seen in meta-anaphase cells. Trivalents were either tandem with the three chromosomes end-to-end or triradial with three chromosome ends forming a single junction. All quadrivalents were tandem. Figure eight shows a cell containing an N-shaped quadrivalent, two open bivalents, and a ℓ-shaped tandem trivalent. The trivalent and quadrivalent are oriented so that adjacent chromosomes face opposite poles. This was characteristic of tandem trivalents and quadrivalents in the haploid. The total numbers of bivalents, trivalents and quadrivalents which were observed are recorded in Table I.

Table I

Bivalents, trivalents and quadrivalents in meta-anaphase cells

Association	Frequency
Bivalents:	
Open	3317
Closed	54
Trivalents:	
Tandem	111
Triradial	16
Quadrivalents:	
Tandem	3
TOTAL	3501

At meta-anaphase the maximum number of bivalents observed within a single cell was seven. Figure 12 shows a cell with six bivalents, figures 11 and 14 show cells with five. Meta-anaphase cells were classified according to the number of bivalents they contained. The number of bivalents per cell varied from zero to seven, making eight classes. Since all cells contained 21 chromosomes the number of univalents was small when the number of bivalents was large. The number of cells observed in each of the bivalent classes is shown in Table II.

Table II

Number of cells in each of the bivalent classes

Class biv. - univ.*	Frequency
0 - 21	325
1 - 19	527
2 - 17	582
3 - 15	356
4 - 13	130
5 - 11	32
6 - 9	8
7 - 7	2
TOTAL	1962

* Abbreviation for bivalents and univalents.

In most of the meta-anaphase cells there was a clear distribution of chromosomes to the plate and poles and reliable determinations of the number of chromosomes in each group were possible. In the remainder grouping was usually evident, but the number of chromosomes in each group was recorded as "undetermined" in order to avoid arbitrarily assigning a chromosome, or chromosomes, to one of two possible groups. Excepting figures 18 and 24 the distribution in all meta-anaphase cells shown was undetermined. Table III gives the number of cells in which the distribution was undetermined for each of the bivalent classes. For the class containing no bivalents there was a large number of cells in which the distribution was undetermined. Since these cells contained no bivalents there is a possibility that they were at diakinesis and incorrectly classified. Undetermined cells in other classes may have been at a stage comparable to prometaphase in normal meiosis. The class of cells containing no bivalents, and also the "undetermined" cells in other classes are not included in later analyses; the possibility of introducing error through incorrect classification is therefore reduced.

Table III

Number of undetermined cells in each of the bivalent classes

Class biv. - univ.	Distribution determined	Distribution undetermined
0 - 21	130	195
1 - 19	432	95
2 - 17	503	79
3 - 15	326	30
4 - 13	123	7
5 - 11	30	2
6 - 9	7	1
7 - 7	2	0
TOTAL	1553	409

Univalents at the plate were recorded for all meta-anaphase cells, including those in which the distribution was undetermined. The equatorial plate was considered to extend from one side of the cell to the other, and about two-thirds the length of a bivalent daughter-chromosome in the direction of each pole. For many univalents there was no difficulty in recording them as at the plate (figs. 7, 9, 18, 19) but for others the decision was often arbitrary

(figs. 6, 13, 15, 21, 23). Although no record was kept, it is probable that the proportion of arbitrary decisions was about the same for all bivalent classes. A maximum of four univalents was recorded as being at the meta-anaphase plate. The data concerning the number of univalents at the plate are given in Table IV. It can be seen from the table that there is a univalent at the plate for approximately every three cells, or, that there is approximately 0.3 univalents per cell at the plate.

Table IV

Number of univalents at the plate and frequency of cells containing them

Number of univalents at plate	Frequency (cells)	Total univalents at plate
0	1393	0
1	460	460
2	88	176
3	18	54
4	3	12
TOTAL	1962	702

Figures one to three, which have been selected to show the cytological features of normal meiosis in wheat, are of monosomic cells of the same anther. Each contains a univalent (arrow in fig. 3). It is interesting to note that contraction has apparently continued from prometaphase to early anaphase. It is difficult to judge whether the haploid meta-anaphase chromosomes are hypercontracted as has been previously reported. (It is well known that contraction in mitotic cells continues to the end of anaphase, reaching its maximum at "tassement polaire").

End-to-end (e-e) and side-by-side (s-s) associations were also seen in meta-anaphase cells. They were found usually at the poles. End-to-end associations were formed of either two or three chromosomes; the latter, termed e-e-e associations, were less frequently seen. An e-e association is shown at the lower pole in figure seven. Without exception s-s associations were formed of two chromosomes. They are shown at the poles in figures 6, 9, 11, 14 and 15. Many cells contained more than one e-e or s-s association. The frequencies of cells with one or more associations are given in Table V. It can be seen that the maximum number of s-s associations within a single cell was five; this was seen in a cell containing one bivalent.

Table V

Number of cells containing one or more e-e, e-e-e and s-s associations

Associations per cell		Frequency	Total chromosomes associated
1	e-e	882	1764
2	e-e	528	2112
1	e-e-e	135	405
	TOTAL	1545	4281
1	s-s	347	694
2	s-s	113	452
3	s-s	43	258
4	s-s	8	64
5	s-s	1	10
	TOTAL	512	1478

In a very few cells e-e and s-s associations were seen in the equatorial region. In these the e-e associations were oriented parallel with the polar axis (figs. 16-17) and the s-s associations perpendicular to the polar axis (fig. 24).

In certain other cells the orientation of the univalents in the equatorial region suggested very strongly that they were daughter-halves of s-s associations which had previously separated at the plate. This is shown very clearly in figure 23 where three, and perhaps four, pairs of chromosomes are oriented more or less symmetrically with respect to the meta-anaphase plate. Other cells show evidence that this may have occurred (figs. 6, 10, 21, 22).

The possibility that e-e and s-s associations may divide at the plate was not realized at the time the method of scoring was devised. It was not realized, in fact, until the analysis of chromosome movement indicated that such a process must occur. From subsequent examinations on the haploid cells there is strong indication that the majority of "undetermined" cells contain dividing e-e and s-s associations.

The distribution of chromosomes to the poles in meta-anaphase cells was also recorded. It was assumed that the daughter-halves of bivalents would be distributed equally to the poles as they are in normal anaphase (fig. 3). It was also assumed that the univalents at the plate would be distributed randomly. For cells with ten chromosomes distributed to each pole and with a univalent at the plate the distribution was recorded as ten and eleven. For cells with unequal distributions, it was assumed that the univa-

lent would move to the pole containing more chromosomes as often as to the pole containing fewer chromosomes. For the 85 cells containing two univalents at the plate it was assumed that they would distribute one to each pole in half the cases and two to a single pole in the remainder. The principle of randomness in univalent movement to the poles was used throughout.

Although the methods employed in recording the chromosome distribution at meta-anaphase have the defect that they anticipate events which have yet to happen, they are made necessary in order to compare events at this stage in this haploid with those at the stage usually designated as "anaphase" in other haploids. It is important to notice that if there are errors resulting from arbitrarily assigning univalents to one or other of the poles, the error will favor randomness in the recorded distribution, since randomness in univalent movement is assumed throughout.

The meta-anaphase distribution as recorded is shown in Table VI. In cells containing one or more bivalents the number of univalents free to move randomly, if indeed they do so, is reduced. Randomness in univalent movement is examined and analyzed in a later section. It should be emphasized that in the recording of chromosome distribution arbitrary methods were used in approximately 30% of the cells

(Table IV) and in these it was assumed that the movement of univalents from the plate was random.

Table VI

Chromosome distribution in meta-anaphase cells

Distribution	Frequency
10 - 11	657
9 - 12	485
8 - 13	266
7 - 14	93
6 - 15	32
5 - 16	8
4 - 17	3
Others	0
Subtotal	1544
Undetermined	409
TOTAL	1953*

* The 9 cells containing 6 and 7 bivalents (see Table III) are omitted in the analysis. These would bring the total to 1962.

Early telophase was sharply delineated from the termination of meta-anaphase by the fact that all chromosomes appeared longitudinally double (cf. Literature Review). The chromosomes were more closely grouped at the poles and it was not possible to count the chromosomes in the polar groups. Only 31 poorly stained cells were observed. In all cells there were univalents at the "plate" oriented in the equatorial plane, and these could be counted. As many as seven were seen in a single cell. The telophase cells, grouped according to the number of univalents at the plate, are recorded in Table VII. It can be seen that the number of univalents at the plate per cell is much higher than at meta-anaphase. At telophase there are approximately four per cell; there was less than one per cell at meta-anaphase (Table IV).

As telophase proceeded the polar groups, often of unequal sizes, became diffuse and circular in outline, forming typical resting nuclei. In many cells micronuclei were formed.

Only a few cells were available for observation at second meiosis. In all cells at least one cell wall had formed. No restituted MI cells were seen. In a few cases the microsporocyte was divided into three or more compartments

of varying sizes. As few as three chromosomes were seen dividing normally within a small cell. Tetrads, when formed, were often composed of two large and two small nuclei. Micronuclei were commonly seen, and in some were separated by a cell wall and appeared as minute cells.

Pollen was irregular in shape. Only a few grains were seen which appeared to be of normal size.

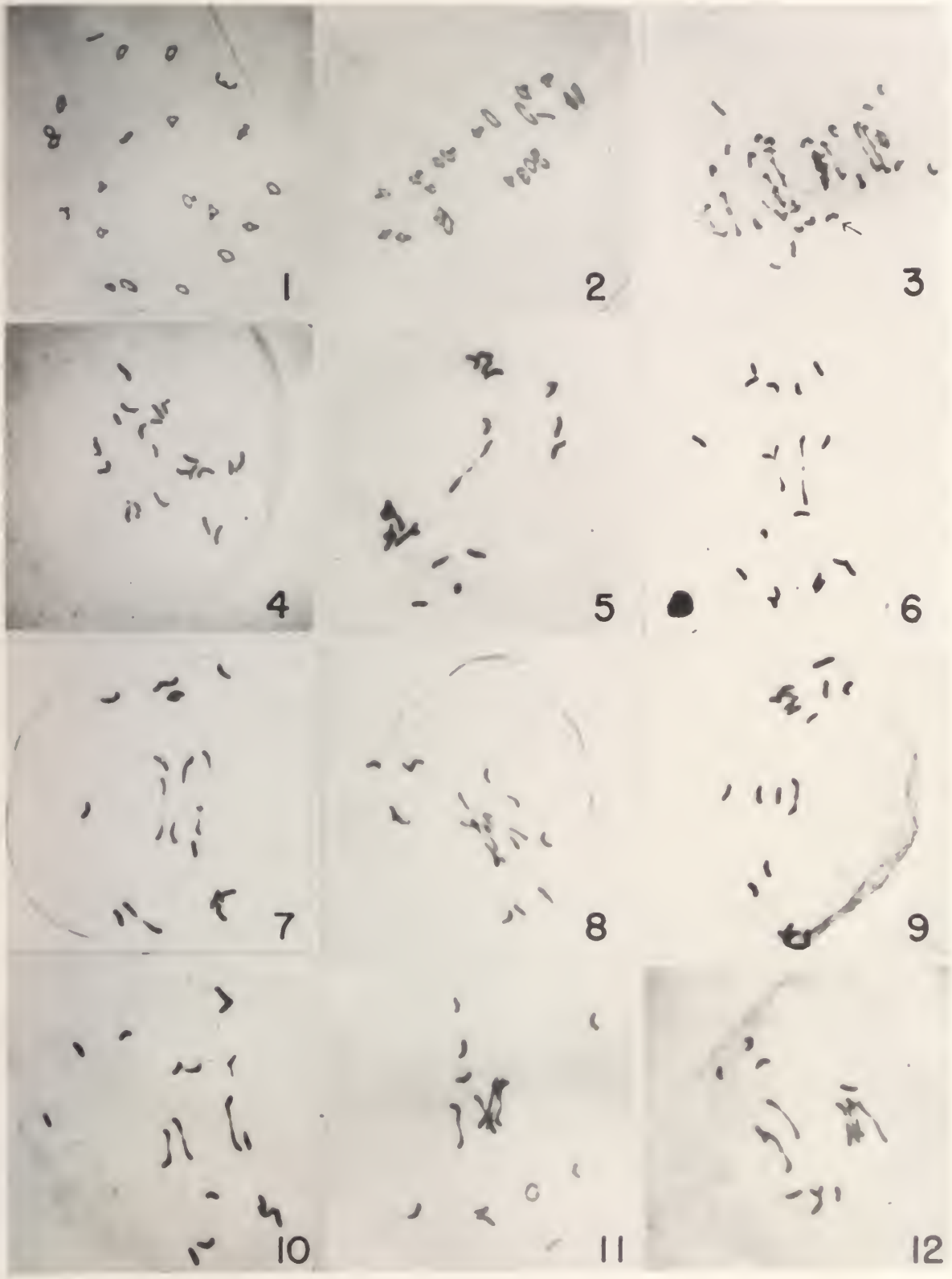
Table VII

Univalents at the plate in telophase cells

Number of univalents	Frequency (cells)	Total univalents
2	10	20
3	7	21
4	6	24
5	4	20
6	3	18
7	1	7
TOTAL	31	110

Description of figures:

Figs. 1-3 of monosomic meiosis showing prometaphase, metaphase and early anaphase. Figs. 4-24 of haploid meiosis, fig. 4 of late prophase, figs. 5-24 of meta-anaphase. Bivalents at plate in figs. 5-15, 18-21 and 24. Univalents at plate in figs. 7, 9, 15, 18, 19. S-s association at plate fig. 24 and divided at plate figs. 21-23. S-s association at poles figs. 6, 9, 11, 13-16, 18, 20, 23-24. E-e association at plate fig. 16-17; at poles figs. 7, 12, 18, 20.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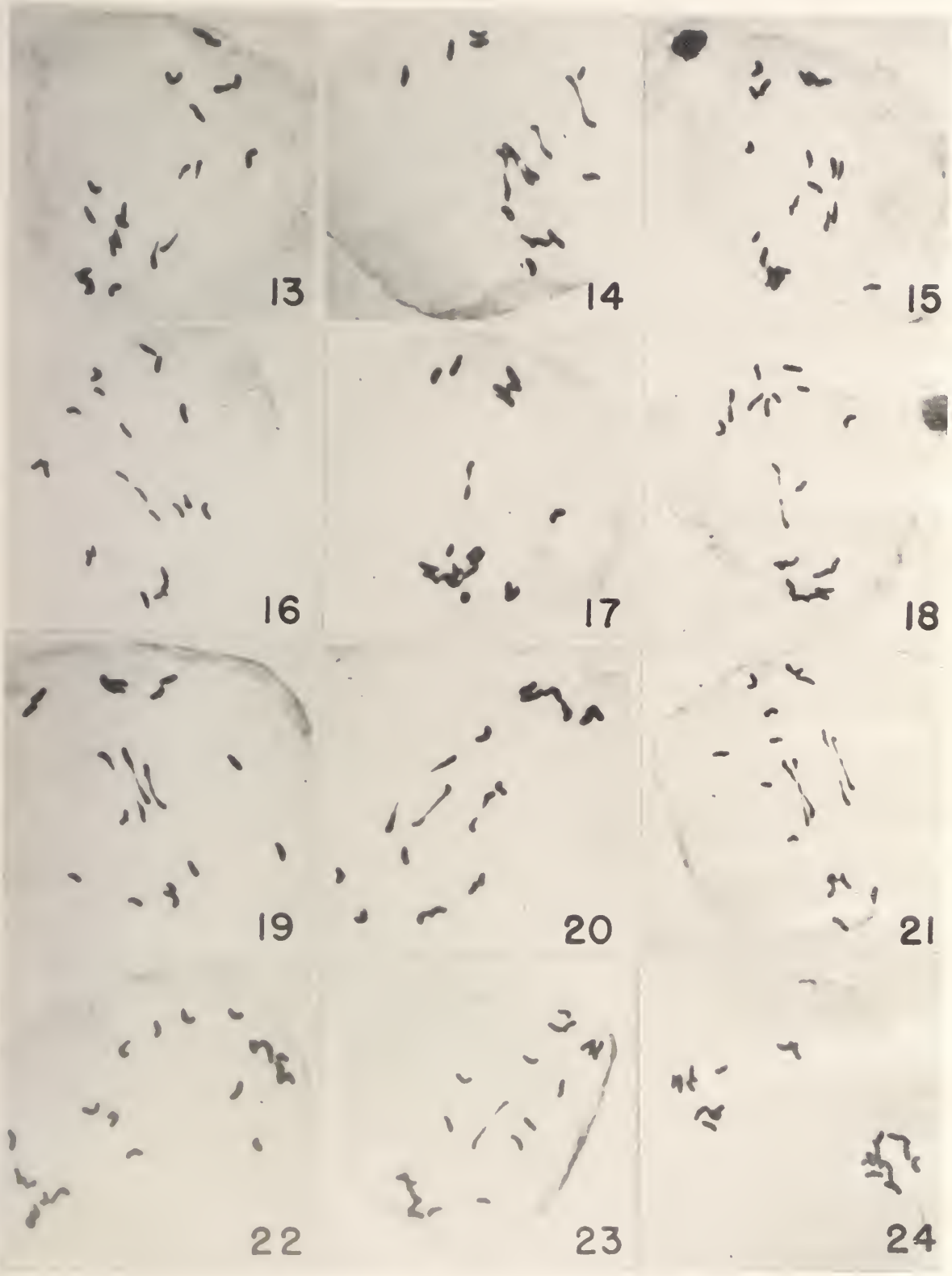
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ANALYSIS

To determine if the distribution of the univalents to the poles at meta-anaphase is random, and to compare univalent activity in this haploid with others reported, allowances must necessarily be made for the large number of bivalents formed in this haploid. For each class of cells containing zero to five bivalents, random distributions were calculated for the number of univalents appropriate to each class and corrections were made to account for the number of bivalents present. Calculations of expected distributions, based on the actual number of cells observed in each class, were then made. No calculations were made for cells containing six and seven bivalents since there were only nine cells in these two classes. For each class of cells it was assumed that the bivalent halves would distribute equally, that is, one daughter chromosome to each pole. In all classes, therefore, the distribution of all 21 chromosomes is treated, as it was in the observational procedure. Random behavior is attributed, however, only to those chromosomes not associated as bivalents. Expected frequencies for all classes were totalled and compared with observed frequencies

using the X^2 method of analysis. This process allowed for treatment of the entire population of 1542 cells and gave greater reliability to the analysis. The calculated and observed data for the analysis are presented in Table VIII.

Table VIII

X^2 analysis of calculated and observed data in 1542 haploid cells

	Calculated frequencies						Total calculated	Observed	$\frac{(o-c)^2}{c}$
No. bivs.	0	1	2	3	4	5	c	0	
Distribution									
10 - 11	44	152	186	128	51	13	574	657	12.00
9 - 12	36	125	149	100	38	10	458	485	1.59
8 - 13	25	83	95	60	21	5	289	266	1.83
7 - 14	14	45	47	27	9	2	144	93	18.06
6 - 15	7	19	18	9	3	0	56	32	10.29
5 - 16	3	6	5	2	1	0	17	8	4.76
4 - 17	1	2	1	0	0	0	4	3	0.25
3 - 18	0	0	0	0	0	0	0	0	0.00
Total No. cells	130	432	503	326	123	30	1542	1544	X^2=48.78

A X^2 value smaller than 14.07 would have supported the assumption that the movement of chromosomes not associated as bivalents is random. These results indicate that the distribution of the univalents to the poles is not random and consequently, the factors which interfere with randomness in univalent distribution must be discovered.

In many cells where e-e and s-s associations occur it might be expected that these will interfere with the random distribution of the chromosomes. If it is assumed that every cell with no bivalents contains one such association the number of effectively separating units is reduced to 20, but the final frequency, excepting for the smallest category (cells with 20-1 distribution) remains unchanged. Since only $1/2^{10}$ cells fall in this category, the effect of one paired association on the distribution is negligible.

It is generally accepted that chromosome homology is an influential factor in determining the movements of the chromosomes during prophase of meiosis. The following statement is taken from Catcheside (7), "According to our present knowledge, chromosome association at meiosis is maintained, subsequent to pachytene, by chiasmata and not by chromosome attraction (homology). In fact, the homologous portions previously paired appear to exert a mutual repulsion. The

chiasmata are formed only in regions previously paired, while pairing at and prior to pachytene is due solely to homologies existing between the parts of the chromosomes paired." Similar statements have been made by Darlington (9) and others (6, 16, 19, 25). It is considered that the pairing properties (attraction and repulsion) of the chromosomes are specific to their constituent particles (chromomeres), so that the whole chromosome does not act as a unit. It is well known that crossing-over and chiasma formation occur less frequently between genes situated close together on a chromosome. Considered in terms of chromosome length this means that chiasmata are less likely to occur when the paired segment is short. Quoting again from Catcheside, "In general, chiasma frequency is a function of the length of paired chromosome." A statement of these generally accepted principles is necessary at this point because they are the premises on which the remainder of the analysis depends.

In normal meiosis homologous chromosomes almost invariably pair and separate equationally* to the poles. Chromosome movement is stereotyped; meiosis is regular. In the haploid where bivalent formation and separation is

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* that is, with one daughter chromosome moving to each pole. This meaning is preserved in the remainder of the study.

irregular, there is a possibility, in cells containing few or no bivalents, that homology or partial homology between unpaired univalents may still play an active role in determining univalent behavior at meta-anaphase. To investigate this possibility attention was directed to the univalent associations (e-e and s-s) in the hope of revealing a correlation between their behavior and that of the bivalents.

Although there was no evidence of chiasmata in either the e-e or s-s associations it is possible that chromosome homology was concerned in bringing the chromosomes together. Since up to seven bivalents were observed in single meta-anaphase cells, and since homology must be constant from cell to cell, it is certain that where there are few or no bivalents there are univalents with segments homologous to segments of other univalents in the same cell. If the incidence of e-e or s-s associations, or both, is found to be higher in these cells it may be suggested that homology is concerned in bringing the chromosomes together. If, on the other hand, the formation of e-e and s-s associations is unaffected by fluctuations in the number of bivalents, it may be concluded that chromosome homology is not concerned with the formation of these associations.

In a cell where no bivalents are formed there are 21 univalents which are free to enter into e-e and s-s associations; in a cell with five bivalents this number is reduced to eleven, or approximately one-half. Is the occurrence of a single association of equal significance in both cells? Clearly, if association is a random process, not conditioned by chromosome homology, it is more likely to occur in the cell with 21 chromosomes free to associate. Comparisons were made, therefore, on the proportion of the total available univalents found actually associated in each class of cells. The proportions, in per cent, of univalents associated end-to-end, for each class of cells containing zero to five bivalents are presented in Table IX. The data show that there is no clear correlation between the number of e-e associations and the number of bivalents in the cell. When there are five bivalents formed the homology is considerably depleted yet there is no corresponding decrease in the proportion of univalents forming e-e associations. It is concluded that e-e associations result from some factor other than chromosome homology.

The possibility, proposed earlier, that chromosome homology may influence the behavior of chromosomes not associated as bivalents, has been eliminated so far as e-e association is concerned.

Table IX

Proportions of univalents forming e-e associations in cells of classes containing zero to five bivalents

Class biv.-univ.	Frequency	Total univalents	Total associations*	Total univalents associated	% of total univalents associated end-to-end
0 - 21	325	6825	326	956	14.0
1 - 19	527	10013	459	1307	13.0
2 - 17	582	9894	457	1255	12.7
3 - 15	356	5340	216	530	9.9
4 - 13	130	1690	69	189	11.2
5 - 11	32	352	16	40	9.1
TOTALS	1952	34114	1543*	4277	12.5

* Including e-e and e-e-e associations.

* Two associations in the 6-bivalent class bring the total to 1545.

The same methods were used in studying the relationship between s-s association and bivalent formation. The data are shown in Table X; they show that the frequency of s-s associations varies <u>inversely</u> with the number of bivalents

Table X

Proportions of univalents forming s-s associations in cells of classes containing zero to five bivalents

Class biv.-univ.	Frequency (cells)	Total univalents	Total associations	Total univalents associated	% of total univalents associated side-by-side
0 - 21	325	6825	163	326	4.8
1 - 19	527	10013	247	494	4.9
2 - 17	582	9894	211	422	4.3
3 - 15	356	5340	92	184	3.5
4 - 13	130	1690	25	50	3.0
5 - 11	32	352	1	2	0.6
TOTALS	1952	34114	739	1478	4.3

present. If both types of association, bivalent and s-s, result from chromosome homology, which is constant from cell to cell, this is the relationship which would be expected. The possibility which was proposed, that chromosome homology may influence the behavior of chromosomes not associated as bivalents, becomes a reality in the case of s-s associations, for here the chromosomes are associated as a consequence of homology.

How does s-s association come about? It is reasonable to suppose that during early prophase there is pairing of all, or nearly all, the homologous chromosomes or chromosome segments. The lengths of the paired segments would vary from pair to pair and from cell to cell. Chiasmata, when they form, link the paired chromosomes together as bivalents. But since crossovers occur at random the chance of a chiasma within any given paired segment would be determined largely by its length (cf. Catcheside, 7). It is suggested that when crossing-over and chiasma formation fail an s-s association is produced. On this hypothesis s-s associations are similar to bivalents during early prophase except that they are not bound together by chiasmata. Through late prophase and at meta-anaphase it would be expected that the forces of repulsion which bring about terminalization of chiasmata in the bivalents would also be operating within an s-s association. Assuming for the moment that this is true, what would be the expected effect of chromosome repulsion on the daughter-halves of an s-s association? In normal wheat the chromosomes forming a bivalent are homologous throughout the length of the bivalent. There is sufficient repulsion to terminalize the chiasmata and open the bivalent in the form of a ring. In the bivalents of the haploid where there is typically only one

chiasma, and more particularly in the s-s associations where there is none because of the decrease in the lengths of the paired homologous segments, it would be expected that the repulsion forces would be weaker and that separation would be less regular, with failure of separation occurring in a certain proportion of the associations. An s-s association, if separation fails, would thus be observable at meta-anaphase in the haploid.

This hypothesis, that s-s associations are produced when chiasma formation fails in the paired homologous segments common to two chromosomes, and that separation also fails in a proportion of these so that they are observable as s-s associations at meta-anaphase, was tested in the following way: A maximum of seven bivalents was observed in two of the 1962 cells studied. It was assumed that homologous pairing sufficient to produce seven bivalents invariably occurred in every cell, and that varying numbers of bivalents and s-s associations were produced depending on the extent of chiasma formation. It was also assumed that a constant proportion of s-s associations failed to separate by meta-anaphase. To obtain a measure of the s-s associations failing to separate the class of cells containing no bivalents was chosen, for here, theoretically, there occur seven s-s associations in every cell. When the figure representing the proportion of

s-s associations failing to separate was determined for this class it was used as a multiple in each of the other classes in order to predict the numbers of s-s associations observable there at meta-anaphase. The calculated and observed values were then compared using the X^2 method of analysis.

In 325 cells containing no bivalents 163 s-s associations were counted at meta-anaphase. These cells contained, theoretically, 325 x 7 = 2275 s-s associations during prophase. Separation failed, therefore, in 163/2275 or 0.072 of the s-s associations. In other classes of cells the number of s-s associations at prophase was obtained by subtracting the number of bivalents present from seven. (Seven is the theoretical sum of bivalents and s-s associations in all cells). This number was then multiplied by 0.072, the proportion of s-s associations which, theoretically, fail to separate by meta-anaphase, in order to predict the number of s-s associations observable at meta-anaphase in each bivalent class. The data and X^2 analysis are presented in Table XI. The hypothesis presented is adequate to meet the experimental facts; a deviation this far from the theoretical would occur in 25% of the tests due to chance alone. The formation of s-s associations through failure of chiasma formation and the separation, before meta-anaphase, of all but 0.072 of them is considered established.

Table XI

X^2 analysis of calculated and observed frequencies of s-s associations at meta-anaphase in cells containing one to five bivalents

Bivalents per cell	S-s assn's. per cell (calculated)	Frequency	Total s-s assn's. (calculated)	S-s assn's. at meta-anaphase (Total x 0.072)	S-s assn's. at meta-anaphase Observed	$\frac{(o-c)^2}{c}$
1	6	527	3162	228	247	1.67
2	5	582	2910	210	211	0.00
3	4	356	1424	102	92	1.00
4	3	130	390	28	25	0.32
5	2	32	64	4	1	2.25

$X^2 = 5.24$

$P = 0.25$

The next step in the search for the factors responsible for non-randomness in univalent distribution was to consider the fate of the daughter-halves of the separating s-s associations. They are found at the poles at meta-anaphase, but have the movements to the poles been random or organized? If random, the only chromosomes in the haploid whose distribution is organized are the bivalents; this hypothesis was tested

earlier and found incorrect. If all s-s associations separate equationally, as do the bivalents, the distribution in all classes of cells would be the same, with seven pairs of chromosomes separating equationally to the poles in every cell; the observation of polar groups of less than seven chromosomes renders this postulate unlikely. It is reasonable to assume that the true situation is somewhere between the two extremes, that the two chromosomes of an s-s association can go to the same pole, but that they are more likely to go to opposite poles.

The method of prediction and testing was used to test the possibility that the two members of an s-s association are more likely to go to opposite poles than to the same pole. To obtain an empirical estimate of the proportion of equationally separating s-s associations the class with the largest number of cells was chosen. This was the class with two bivalents and seventeen univalents. In the determination it was assumed, successively, that two, three, and up to seven pairs of chromosomes were separating equationally and distribution frequencies were calculated for each assumption. The X^2 test was applied to each, and the assumed frequency most closely approximating the observed frequency was selected and used for the empirical estimation. The X^2- and P-values are given in Table XII.

Table XII

X^2 and P values of a series of calculated distribution frequencies tested against the frequency observed in the class of cells containing two bivalents

Calculated frequencies				
Pairs separating equationally		Univalents separating randomly	X^2	P
2	-	17	29.14	0.00
3	-	15	16.98	0.01
4	-	13	6.77	0.30
5	-	11	2.65	0.60
6	-	9	6.20	0.20
7	-	7	91.88	0.00

They show, first, that this class of cells does not give the distribution expected if the movement of the univalents is random, and second, that the distribution observed compares most closely with that expected for five equationally-separating pairs. The obvious conclusion is that in addition to the two bivalents present in the cells of this class there are three more chromosome pairs whose separation is equational. On the hypothesis being tested these are s-s associations. There are,

theoretically, five s-s associations in each of the cells of this class; three of them, on the average, are separating equationally. This suggests that the proportion of equationally separating s-s associations should be set at three out of five, or 0.6, but for simplicity in later calculations the proportion is approximated to 0.5, or one-half.

From comparing a series of calculated distributions with the distribution observed for cells containing two bivalents it is therefore concluded, that on the average, approximately half of the s-s associations separate equationally. Applying this concept to single s-s associations it means that distribution is equational in 50% of the separations and random in the remaining 50%. But of the random separations, half of these are also equational. The sum of this is that an s-s association separates with one daughter chromosome moving to each pole in 75% of the cases, and with both daughters moving to a single pole in the remaining 25% of the separations. Considering for a moment the class of cells with two bivalents and five s-s associations: it has been concluded that one-half the s-s associations separate equationally; there is no evidence, at this point, to suggest that in all cells it is the same s-s associations which give this type of separation. The probable condition, that all s-s associations may separate

equationally in some cells and all randomly in other cells, has not been excluded.

Based on the assumption that the s-s associations separate equationally in half the cases and randomly in the other half, distribution frequencies were calculated for all cells of all classes, that is, the entire population of cells whose distribution was recorded, in order to check the validity of the assumption. For example, in the class containing two bivalents and five s-s associations it was considered that distribution of the s-s associations would be as shown in Table XIII. From the table it can be seen that the proportions of equational and random distributions are equal. The convenience of approximating the empirical determination at one-half, as stated earlier, can also be seen. The next step was to reclassify the cells in this group making adjustments for the total numbers of chromosome pairs, including bivalents, with equational separation. To illustrate, 1/32 of the cells with five s-s associations separating equationally were considered as though they contained seven bivalents. The 503 cells containing two bivalents, reclassified, are listed in Table XIV. It can be seen that the numbers of s-s associations separating equationally have been added to the number of bivalents, and those distributing randomly have been included with the univalents. The total numbers of cells in each of the other

Table XIII

Calculations used in determining the distribution of the five s-s associations in cells containing two bivalents

S-s associations distributing equationally	S-s associations distributing randomly	Proportion of total cells
5	0	1/32
4	1	5/32
3	2	10/32
2	3	10/32
1	4	5/32
0	5	1/32

Table XIV

Cells containing two bivalents reclassified according to the numbers of chromosome pairs separating equationally

Number of pairs separating equationally	Number of univalents	Frequency
7	7	16
6	9	79*
5	11	157
4	13	157
3	15	78
2	17	16
		503

* It was necessary to add a cell to one of the groups to maintain the total of 503.

bivalent classes were similarly reclassified. Table XV shows the reclassifications of all classes of cells.

Table XV

Cells of all bivalent classes reclassified according to the number of chromosome pairs separating equationally

Pairs separating equationally	0	1	2	3	4	5	6	7	Total
Previous classes biv.-univ.									
0 - 21	1	7	21	36	36	21	7	1	130
1 - 19	-	7	40	101	136	101	40	7	432
2 - 17	-	-	16	79	157	157	78	16	503
3 - 15	-	-	-	20	82	122	82	20	326
4 - 13	-	-	-	-	16	46	46	15	123
5 - 11	-	-	-	-	-	7	16	7	30
TOTAL	1	14	77	236	427	454	269	66	1544

In accordance with the assumption that one-half the s-s separations are equational and the other half random, the number of equationally separating chromosome pairs in each class

of cells was reassessed. The re-assessment when totalled gave eight classes of cells containing from zero to seven equationally separating pairs. For instance, there are 464 cells containing five pairs of chromosomes whose separation is equational; these include cells from all former classes containing zero to five bivalents, but the total of the bivalents and the estimated number of equationally-separating s-s associations is five in all cells of the group.

The validity of the assumption that separation is equational for approximately one-half the s-s associations was next tested. Using the reclassification shown in Table XV, the distribution frequencies were calculated for all eight classes of cells. These are recorded in Table XVI. The right-hand column shows the calculated frequencies for all cells, based on the assumptions outlined above. These were compared with the distribution frequencies actually observed, using the X^2 test for goodness of fit. The results of the comparison are shown in Table XVII.

The P-value of 0.30 indicates that the observed distribution is satisfactorily explained on the assumption that an average of one-half the s-s associations separate equationally. It will be noticed that the deviation between observed and calculated frequencies occurs almost entirely in

Table XVI

Calculated distribution frequencies following reclassification

Pairs separating equationally	0	1	2	3	4	5	6	7	
Univalents distributing randomly	21	19	17	15	13	11	9	7	Total
Distribution									
10 - 11	1	5	28	93	178	202	124	28	659
9 - 12	0	4	23	72	134	145	85	19	482
8 - 13	0	3	15	43	75	74	39	9	258
7 - 14	0	1	7	18	30	26	14	5	101
6 - 15	0	1	3	7	8	7	7	5	38
5 - 16	0	0	1	2	2	0	0	0	5
4 - 17	0	0	0	1	0	0	0	0	1
TOTAL	1	14	77	236	427	454	269	66	1544

Table XVII

X^2 test of calculated and observed distribution frequencies

Chromosome distribution	Frequencies calculated	Frequencies observed	$\frac{(o-c)^2}{c}$
10 - 11	659	657	0.01
9 - 11	482	485	0.02
8 - 13	258	266	0.25
7 - 14	101	93	0.64
6 - 15	38	32	0.95
5 - 16	5	8	1.80
4 - 17	1	3	4.00
TOTAL	1544		$X^2 = 7.67$

P = 0.30

the 5-16 and 4-17 distribution classes. This may be because of the relatively small numbers of cells in these classes. The comparison for the rest of the classes is very close. (P = between .5 and .95). Because the assumption that one-half s-s separations are equational and the remainder are random gives a satisfactory explanation of the observed facts it will be assumed, for the remainder of the analysis, that the assumption is correct.

On the basis of their behavior during prophase and meta-anaphase there are two kinds of chromosomes in the haploid. There are those that pair and give rise to bivalent and s-s associations, and there are those that do not pair. For the first group, separation is equational for all bivalents and one-half the s-s associations, the daughter-halves of the remaining s-s associations move randomly to the poles. For the second group there is separation to the poles but the distribution is characteristically random. To distinguish the two kinds of chromosomes in subsequent discussion those belonging to the group, having homologues, will be referred to as "A-chromosomes" and the remainder, having no homologues, will be referred to as "P-chromosomes".

The behavior of the A-chromosomes has been attributed to chromosome homology; their movements during prophase are directed by forces of attraction and repulsion; their response to these forces of attraction and repulsion is active. The P-chromosomes, having no homologue, do not respond to the forces of attraction and repulsion which are coincident with homologous association; as far as these forces are concerned the P-chromosomes are passive. The abbreviates A, for active, and P, for passive, have been chosen to represent this concept in the following discussion.

A-chromosomes form s-s associations during prophase and by meta-anaphase most of these have separated and the daughter halves, now termed A-univalents, have moved to the poles. Accordingly, the polar groups at meta-anaphase are composed of both A- and P-univalents. It is important to note that the proportions of A- and P-univalents are changed with every change in the number of bivalents per cell. These changes are tabulated in Table XVIII. With more bivalents per cell the proportion of A univalents decreases and the proportion of P univalents increases. It was found earlier (Table X) that the proportion of the total univalents forming s-s associations varies inversely with the number of bivalents present in the cell. This relationship is shown again in Table XVIII; it can be seen that both the number and the proportion of A univalents vary inversely with the number of bivalents found in the cell. It was also found earlier (Table IX) that the proportion of the total univalents forming e-e associations was constant from cell to cell. Referring, analagously, to Table XVIII, this relationship can be seen in the right-hand column. The conclusion reached earlier, that e-e association is a random process unconditioned by chromosome homology, can now be restated: both A and P univalents enter randomly into e-e association.

Table XVIII

The proportions of A and P univalents in meta-anaphase cells containing zero to seven bivalents

Class biv.-univ.	Number of A-univs.	Number of P-univs.	Total A.and P univs.	Proportion of A-univs.	Proportion of P-univs.	Proportion A and P univ s.
0 - 21	14	7	21	0.67	0.33	1.00
1 - 19	12	7	19	0.63	0.37	1.00
2 - 17	10	7	17	0.59	0.41	1.00
3 - 15	8	7	15	0.53	0.47	1.00
4 - 13	6	7	13	0.46	0.54	1.00
5 - 11	4	7	11	0.36	0.64	1.00
6 - 9	2	7	9	0.22	0.78	1.00
7 - 7	0	7	7	0.00	1.00	1.00

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The next step in the analysis was to consider the incidence of univalents at the plate. As with s-s and e-e associations the proportion of the total univalents found at the plate was determined for each class of cells. These are shown in Table XIX.

Table XIX

The proportion of total univalents at the plate in each class of cells containing from one to five bivalents

Class biv.-univ.	Frequency (cells)	Total univalents	Total univalents at plate	% of total at plate
1 - 19	527	10013	185	1.8
2 - 17	582	9894	232	2.3
3 - 15	356	5340	191	3.6
4 - 13	130	1690	78	4.6
5 - 11	32	352	16	4.5
TOTALS	1627	27289	702	2.6

With the exception of the last (and smallest) bivalent class the proportion of univalents at the plate increases directly with the increase in the number of bivalents per cell. Failure of complete agreement is considered to result from the small number of cells in the five-bivalent class. Referring to Table XVIII it can be seen that the proportion of P-univalents per cell also increases with increasing bivalents.

Clearly, if the univalents found at the plate are P-univalents the relationship becomes understandable, for this is the only column in which the proportion of chromosomes increases directly with increasing bivalents. This can be verified in another way. From Table XVIII it can be seen that the number of P-univalents from class to class remains unchanged at seven per cell. It should be expected, therefore, if P-univalents only occur at the plate, that the absolute number of univalents at the plate would remain unchanged from class to class. Table XX gives the average number of univalents at the plate for cells of each of five classes containing from one to five bivalents. Here again, with the exception of the last and smallest class, there is a linear relationship between univalents at the plate and bivalents. As before the discrepancy in the last class is considered due to the small number of cells in this class. This relationship is contrary to expectations. It had been expected that the absolute number of univalents at the plate would remain unchanged from class to class. Nor can the agreement be improved if A-univalents as well as P-univalents are considered to come to the plate, for this would entail an expected decrease in univalents at the plate with increasing bivalents, and the discrepancy between expected and observed values would be larger.

The conclusion that only P-univalents occur at the plate is therefore retained, with the provision that their increasing numbers in cells with more bivalents be explained.

Table XX

The average number of univalents at the plate in cells of classes containing from one to five bivalents

Class biv.-univ.	Frequency (cells)	Total univalents at plate	Univalents at plate per cell
1 - 19	527	185	0.35
2 - 17	582	232	0.40
3 - 15	356	191	0.54
4 - 13	130	78	0.60
5 - 11	32	16	0.50

To further examine the behavior of univalents in the haploid a part of the analysis was concerned with determining the time of arrival of P-univalents at the plate. During the transition from prophase to meta-anaphase the chromosomes move either to the plate or the poles. But it is not known if

the position of a univalent at the plate is fixed at this time. A method was devised whereby the time of arrival of univalents at the plate could be determined.

It will be recalled that for each class of cells containing bivalents the number of bivalents with chiasmata and with stretched chromatic threads was recorded. For the class of cells containing one bivalent there were two categories, one in which the chiasma was optically visible and one in which it was no longer detectable. Similarly, for cells with two bivalents there were three categories, depending on whether both, one or none of the bivalents showed chiasmata. Cells in which all bivalents have chiasmata are at an earlier stage in meta-anaphase than those in which no chiasmata are visible. For every class of cells it was possible, therefore, to divide the total number of cells into two groups, early and late. For example, cells containing two bivalents each with a chiasma were classified "early", those with no chiasma "late" and the remainder, with one bivalent with a chiasma, were excluded from the classification. The same principle was applied in classes containing other numbers of bivalents. For each of the early and late groups of cells the proportion of total univalents at the plate was calculated. These are presented in Table XXI. It can be seen that there is no increase in the proportion of

univalents at the plate through this portion of meta-anaphase. It is concluded that the position of a univalent at the plate is determined by the time meta-anaphase begins. It can now be stated that the movements of bivalents and P-univalents to the plate and of A- and P-univalents to the poles occur during the transition from prophase to meta-anaphase.

Table XXI

Proportion of total univalents at the plate in cells classified as early and late

Class	Frequency (cells)	Total univalents	Number at plate	% of total at plate
Early	394	6764	222	3.3
Late	732	12282	355	2.9

It was shown (Table III) that in each of the bivalent classes there was a number of cells in which the grouping of the chromosomes, at the plate and poles, could not be accurately distinguished. These cells were listed as "undetermined". For convenience in later discussion this table is

now revised to show the proportion of undetermined cells in each bivalent class. Cells containing no bivalents are here omitted, because of the possibility that cells at diakinesis may have been erroneously included with the group. The few cells containing six and seven bivalents are omitted also. The revised data are given in Table XXII. There is a decrease in the proportion of cells "undetermined" as the number of bivalents increases. It can equally be stated that with increasing numbers of bivalents there is an increase in the proportion of cells with clear grouping. A possible relationship between this observation and the factors concerned with chromosome movement will be considered in the discussion.

Table XXII

The proportion of cells in classes containing one to five bivalents in which chromosome grouping was undetermined

Class biv.-univ.	Frequency (cells)	Number undetermined	Proportion undetermined
1 - 19	527	95	0.18
2 - 17	582	79	0.14
3 - 15	356	30	0.08
4 - 13	130	7	0.05
5 - 11	32	2	0.06

REVIEW OF ANALYSIS

Method

The method of observation, assumption, prediction and verification was used throughout the analysis. The initial observation was non-random distribution of the univalents in meta-anaphase cells. A search was made for the possible factor or factors causing the deviation from randomness in univalent distribution. Chromosome homology was found to be concerned in the forming of s-s associations; on the premise that homology determines chromosome behavior during prophase it was assumed that it also determined the deviation from randomness in univalent distribition at meta-anaphase. A hypothetical sequence of events was outlined describing the events during prophase and this, coupled with an observed relationship taken from one group of cells, was used to predict correctly the numbers of s-s associations at meta-anaphase for all bivalent groups. The hypothetical sequence of events was extended to include meta-anaphase. A second observation,

taken from one of the bivalent classes, was used to predict correctly the distribution observed in all meta-anaphase cells of the haploid.

The hypothesis, tested and found satisfactory at two separate steps, was considered established and led to the classification of the chromosomes into two groups. Significant facts for discussion were revealed concerning the number of bivalents in the cell and its relation to:

(i) the proportion of univalents at the plate; and
(ii) the proportion of cells in which distribution was undetermined.

The time of arrival of the univalents at the plate was also determined.

It is realized that though the empirical determinations have led to a satisfactory hypothesis they may deviate more or less from their true values. They are considered accurate enough to render the outline generally valid.

Validity

The X^2-test does not prove the validity of the postulate or hypothesis being tested; it can indicate if the hypothesis gives a satisfactory explanation of the observed facts and is perhaps more positive when the postulates are invalid.

The hypothesis developed in this analysis would be invalidated if its premises - the theories of homologous attraction, chiasma-formation and repulsion - are shown to be invalid, or if there is a serious defect in its development. Failing these the hypothesis can be confidently employed in discussing the factors determining chromosome movement.

Chromosome Movement

A more comprehensive description can now be given of the movements of the chromosomes of the haploid during the stages comparable to prophase, metaphase and anaphase of normal meiosis.

During early prophase there is pairing of homologous chromosome segments. If chiasmata occur bivalents and occasionally tri- and quadrivalents are produced. If chiasmata do not occur s-s associations are formed. It is estimated that there are fourteen chromosomes which form homologous associations. By late diakinesis the chiasmata in the bivalents are terminalized owing to repulsion between the constituent halves. Repulsion forces are defective in the paired chromosomes of the haploid because of the reduction in total length of paired segments. Separation fails in 7% of the paired chromosomes of s-s associations. Those failing to separate are observable at meta-anaphase. Separation is effective in the remaining s-s associations. The daughter-halves of separating s-s associations move more often to opposite poles than to the same pole; the proportions are estimated at 75 and 25 per cent. When the two members

of an s-s association fail to separate they are occasionally seen in the equatorial region of the cell, oriented parallel to the metaphase plane with their daughter-halves facing the poles. Cytological evidence indicates that they may divide at the equatorial region and that separation precedes that of the bivalents. It was suggested, though without numerical support, that a large proportion of cells in which distribution was undetermined contained separating s-s associations.

It is not known if pairing occurs among the chromosomes which have no homologue. At some time before meta-anaphase there is e-e association of chromosomes belonging to both A- and P-univalent groups. This association is random and not influenced by homology. The events which precede e-e association are unknown. At meta-anaphase e-e associations are found usually at the poles but occasionally they are seen in the equatorial region of the cell. When found at the equator they are oriented parallel to the polar axis.

Univalents are found at the plate during meta-anaphase. Their number increases when there are more bivalents at the plate. The position of a univalent at the plate is determined at the beginning of meta-anaphase.

Their orientation with respect to the polar axis is parallel or perpendicular; less often it is random.

In agreement with previous authors who have commented on the movements of the chromosomes during the period from diakinesis to meta-anaphase it is considered that the time during which the chromosomes move to their meta-anaphase positions is relatively short.

Accurate determinations of the numbers of chromosomes in the polar and equatorial groups can be made in approximately 80% of the meta-anaphase cells. In the remaining 20% grouping is expressed but the number of chromosomes in each group cannot be reliably determined. The proportion of these cells is greatest when the number of bivalents is small.

The separation of bivalents during meta-anaphase is not synchronous. As separation progresses the chiasmata diminish and disappear, leaving the daughter-halves connected by a fine chromatic thread. Spindle-attachment fibres are less often seen than in bivalents of normal cells. The appearance of doubleness typical of normal anaphase chromosomes is not seen in the haploid during meta-anaphase.

At the termination of meta-anaphase all chromosomes appear longitudinally double. The number of univalents at the plate in these cells is much greater than at

meta-anaphase. These appear to be "splitting" at the plate. Through telophase the nuclei and micronuclei are formed and meiosis I is over.

DISCUSSION

The analysis has provided a description of chromosome movements in the haploid during prophase and meta-anaphase. Since the methods of fixing and staining do not allow for direct observation of the spindle its presence and the time at which it begins to operate can only be surmised from the movements and morphology of the chromosomes.

Spindle fibres in living cells have been only recently demonstrated by Inoué (20) although it had been concluded earlier that there was no longer any doubt of the reality of the spindle (46).

It is generally accepted that the spindle brings about the separation of bivalent daughter-halves during normal meiotic anaphase. If this is true there is no reason to doubt that it plays the same role in the haploid. But the exact time at which the spindle becomes active in normal meiosis is not known. To determine this there is the possibility that evidence may be developed through considering the abnormal meiotic behavior in the haploid.

First, if we assume that the spindle is the sole arbiter of chromosome movement in the haploid it must be capable of the following:

(i) acting differentially on A- and P-univalents. (Distribution for one group is random, for the other it is not. Such differential action is conceivable since A- and P-univalents are known to be different);

(ii) controlling simultaneously the movements of chromosomes to the plate and to the poles;

(iii) moving a greater proportion of univalents to the plate when the number of bivalents also being moved to the plate, is large;

(iv) selectively orienting e-e and s-s associations at the plate parallel and perpendicular to the axis of polarity;

(v) separating the s-s daughter-halves and moving them to the poles; and following this,

(vi) separating bivalent daughter-halves and moving them to the poles.

With the exception of (vi), these actions do not fall within the range of behavior usually attributed to the spindle. To extend activities of the spindle to include all chromosome movement in the haploid would require considerable modification of the theories of spindle action.

As the range of activity attributed to the spindle is widened, theories on the mechanism of its action become necessarily more complex. For this reason alone a search for an alternative mechanism is justified.

If it is assumed that the spindle is concerned only with bivalent separation, what alternative mechanisms can be found to account for the movements of the remaining chromosomes in the haploid?

Schrader, in his excellent book on mitosis has reviewed the many hypotheses concerning the movements of the chromosomes (46). In nearly all the hypotheses polar forces, acting during late prophase, and variously described as "polar diffusion centers", "organizing centers" and

"localized regions of activity", have been invoked to explain the formation of the spindle, the organization of the metaphase plate, the movements of the chromosomes, and combinations of these (2, 3, 34, 43, 45, 46, 51, 53). These hypothetical polar forces were found necessary in order to account for the many events which occur during normal cell division. Let us assume that they operate in the haploid also.

Forces of mutual repulsion between paired homologous chromosome segments have been assumed throughout the analysis. Weaker repulsion between all chromosomes irrespective of homology is believed to act during late prophase and metaphase (3, 9, 13, 43, 46, 51). This repulsion ("body repulsion") has been invoked to explain the position and spacing of the chromosomes at the plate in mitosis and meiosis, and at the nuclear periphery during late diakinesis.

It is the author's opinion that polar forces, coupled with chromosome repulsion, can adequately account for all chromosome behavior in the haploid. To do this the forces must meet with certain requirements:

(i) The polar forces must be equally balanced, with maximum influence at the poles and minimum influence at the equator. They must attract

chromosomes to the poles, and the forces of attraction must act on the whole chromosome rather than any specific part.

(ii) Body repulsion must be strongest between chromosomes longitudinally paired (bivalent and s-s associations) and weakest between single chromosomes (univalents and e-e associations).

(iii) The magnitude of the polar forces, though variable in different parts of the cell, mustlie between that of the body repulsions of paired and single chromosomes.

Chromosome behavior in the haploid can now be explained. At late diakinesis the chromosomes are distributed more or less randomly throughout the nucleus because of body repulsion. When the polar forces come into play the univalents, e-e associations and occasionally(7%) the s-s associations move randomly to the nearest pole. The polar attractions overcome the forces of body repulsion. The orientation of the chromosomes to the poles is random since polar attraction is not specific to any part of the chromosome. Bivalents and remaining s-s associations, whose balance between polar attraction and body repulsion

is reversed, move to the plate. Their position at the plate is determined by strong forces of body repulsion, their orientation at the plate is determined by polar attraction, and their morphology by homologous repulsion. Homologous repulsion in s-s associations, when augmented by the forces of polar attraction, causes the s-s daughter-halves to separate and move to the poles. Since this is known to occur in approximately half the s-s associations it must be presumed that the remaining half were separated prior to the action of polar forces due to homology alone. The distribution of these, as for other univalents, is random. Chiasmata prevent separation of bivalent daughter-halves. This is an equilibrium position for univalents and e-e associations which were at the "plate" before the polar forces became active. Their characteristic orientations at the plate result from polar attraction.

There are two observations still to account for:

(i) The greater proportion of undetermined cells when the number of bivalents is small;

(ii) the greater proportion of univalents at the plate when the number of bivalents is large.

In cells with few bivalents there is a greater proportion of chromosomes whose movement to the poles is via the metaphase plate. These are the s-s daughter-halves. They are

released from s-s association and are free to move to the poles only after having previously moved to the metaphase plate. Chromosome distribution in these cells is more complex. In cells with many bivalents there is a greater total movement to the plate, a smaller total movement to the poles, and the distribution, owing to reduced s-s associations, is less complex. These differences account for the variable proportions of undetermined cells with changing numbers of bivalents. When the number of bivalents is large there are fewer separating s-s associations, or separated daughter-halves in the region of the plate, or between it and the poles. If the movement of a univalent chromosome is influenced by that of its neighbors it will move to the plate more often when there are more bivalents, and be carried away from the plate and to the poles more often when there are more s-s associations. All chromosome movement in the haploid is now accounted for.

There is a close parallel between the behavior of s-s associations in the haploid and of mitotic chromosomes during somatic reduction (19, 56, 57). In a discussion of the mechanism of somatic reduction the following statement appears, "_____ In the first place, we are unable to see how the spindle can be considered as a single unit which is the basic of most theories of chromosome mechanics (cf. Schrader). We would like to suggest that there are at least two more or less independent components:

1. a dipolar cytoplasmic orientation possibly characteristic of the cell at any stage; and 2. a nuclear component inherent in and directed by the chromosome or kinetochore thereof. We would further suggest that metaphase organization is primarily a function of the cytoplasmic component whereas anaphase separation is a function of the chromosome itself. _____ (57)". The suggestions proposed then to account for somatic reduction have been used here, with small modifications, to explain chromosome movement in the haploid. The specifications of the polar and chromosomal forces have been, perhaps, more closely approximated.

Compared with the alternative concept that only the spindle is concerned with chromosome movement, the mechanism proposed earlier (57) and modified here has the following advantages:

(i) It allows for chromosome movement when the spindle is absent, and there are numerous instances where this occurs (9, 10, 19, 39, 46, 56, 57).

(ii) It is concerned with the formation of the metaphase plate which, according to Schrader, "is one of the greatest difficulties of all mitotic hypotheses" (46).

(iii) It obviates the apparent reversal of forces - attraction to the plate and repulsion to the poles - which has been especially difficult to explain in terms of a single agency (3, 9, 19, 34, 43, 45, 53, 57).

(iv) It accounts for defective metaphases and accommodates the few reported instances where bipolarity has apparently been lost (9, 48).

(v) It allows for variability in chromosome behavior at a time when much variability has been observed in both plant and animal cells (3, 6, 9, 10, 11, 19, 25, 29, 34, 36, 39, 40, 45, 46, 48, 51, 53, 57).

(vi) It is applicable to normal meiosis and "meiosis" in other haploids, and possibly to mitosis and mitotic variations (41, 47, 52), as previously suggested (57).

SUMMARY

1. Meta-anaphase cells of the haploid were found to contain variable numbers of bivalents at the plate and of univalents, e-e and s-s associations at the plate and poles.

2. A method of scoring was devised whereby the behavior of each of the variables was recorded for every cell.

3. The observational data was treated mathematically and the following relationships were revealed:

   (i) The proportion of univalents to the poles was not random;

   (ii) the proportion of univalents forming e-e associations was not affected by the number of bivalents in the cell;

   (iii) the proportion of univalents forming s-s associations varied inversely with the number of bivalents in the cell;

   (iv) the proportion of univalents at the plate varied directly with the number of bivalents in the cell;

(v) the number of univalents at the plate was higher at telophase than at metaphase.

4. A hypothesis based on the observed relationships and supported by statistical analyses was outlined to describe the sequence of events up to and including meta-anaphase in the haploid. It included:

(i) homologous pairing of chromosome segments during the prophase sufficient to produce seven paired associations in every cell;

(ii) crossing over in some of these to produce bivalents, failure in the remainder to produce s-s associations;

(iii) separation of all but 7% of the s-s associations before meta-anaphase;

(iv) random chromosome attachment at some time during prophase to form e-c associations;

(v) simultaneous movement at the beginning of meta-anaphase of chromosomes to the plate and poles.

5. The observational data were reviewed and the forces determining chromosome movement were discussed. The movements were attributed to agencies of polar attraction and chromosomal repulsion.

6. The action of these agencies in haploid cells was outlined and their application in other cytological situations was suggested.

CONCLUSIONS

With respect to the general problems of haploid cytology the following conclusions may now be drawn:

(i) chromosome distribution is random only for chromosomes with no homologue;

(ii) chromosome distribution is not random for chromosomes capable of forming bivalents or side-by-side associations;

(iii) tandem association is not influenced by chromosome homology;

(iv) side-by-side or "secondary" association is governed by chromosome homology.

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